Hints for Yeast Showdown I: Image Processing

You are encouraged to complete the exercise first without reading these hints. Once you are done, or if you are having trouble, this will break down the exercise into more manageable chunks. It is ok if what you did looks very different from this. In fact, in real life, you will not get hints, so as you read this, ask yourself why the exercise has been broken up into these steps, and what are the advantages (and disadvantages!) of doing the analysis the way we've presented here.

1. To explore the data:

a. Visualize each of the fluorescent channels separately.

i. Load the phase, RFP, BFP, and YFP, the images you took. You can drag them in to MATLAB as we did before or you can use the command imread. Type `doc imread` to figure out how it works.

ii. Look at one of the pictures with `imshow`. Why can't we see anything? Convert all the image arrays to doubles as we did before. This allows us to more easily display and manipulate the image. Now use imshow. Can you see anything? What is the problem? Remember what we did to show our matrix as an image in section 4.2.

iii. Subplots. Just to confirm that they are all the same field of view, we will put them on the same figure in subplots. Show each of the four images as one corner of a 2 by 2 plot. You can leave the last subplot empty.

b. If you overlay the images, what do you expect to see? Overlay them to form a single image with each channel represented by a Red, Green or Blue. Because of camera settings, they are all on different intensity scales, make sure to normalize each image such that each pixel is a value between 0 and 1. Are there any colors that you didn't expect to see? Why did this happen?

Because these images are basically just different ways to view the same picture, we want to store them as such.

i. Normalize each image and store the result to a new variable. You want to rescale the pixel values in the images such that the lowest pixel is 0 and the highest is 1, while preserving the relative intensities of all pixels in between. Which mathematical operations do you need to accomplish this? What do you need to know about the pixel values in each image?

ii. Make a 3D array and store the three (normalized) fluorescence images into it. Call that variable AllChannels; make layer 1 RFP, layer 2 BFP and layer 3 YFP. This array should be 1024 by 1344 by 3.

iii. View the image in false-color using `imshow`. Plot this as the 4th subplot in the 2x2 figure you made for part 1.b.iii. Which colors represent the 1st, 2nd, and 3rd planes by

default? What colors do you see in the image? What do each of the colors mean? Is there a color that you did not expect to see given the experiment described above?

2. Do the pixels that come from RFP or BFP cells have a higher YFP value? What's the mean and standard deviation of each group of pixels?

a. Make a matrix that acts as a mask for the RFP channel using a single threshold value. Convert every pixel that is part of cell into a 1 and every other pixel into a 0. This is called thresholding; it is a very basic type of image processing. View with **`imshow`**.

You can also threshold an image with the built-in function **`im2bw`**. *Figure out how it works by typing* **`doc im2bw.`**

b. What is the mean YFP pixel intensity of the RFP-labelled cells? The standard deviation? The pixels with value 1 in the thresholded image from #8 make up the cells expressing RFP.

c. Repeat the process for the BFP image. What, if anything, can you conclude about the expression of gene X in the two different genetic backgrounds? What might you need to do to have a more rigorous comparison between the two?

Extra 1. Is the difference in YFP significant? Think about how to determine if two distributions are significantly different. What assumptions have to be met for this method to be used? Think especially about independence of samples.

Extra 2. Find the background YFP level for the image (the YFP values from outside the cells). When you take into account the background is the difference still significant?

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Last Updated 8/15/12 by RE.